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ERGOTHIONEINE (2-THIOL-L-HISTIDINE BETAINE = ET) AS AN ANTIMUTAGEN: INTERCEPTION OF DIRECT-ACTING MUTAGENS FORMED FROM NITROSATION OF SPERMIDINE. Zlata Hartman, Philip E. Hartman and Roland A. Owens, Department of Biology, The Johns Hopkins University, Baltimore, MD 21218.

Ergothioneine (ET) is exclusively synthesized by microbes and concentrated, for example, in conidia of Neurospora. ET is avidly accumulated by higher plants and animals where it constitutes a highly conserved molecular species (Melville 1958 Vit. Hormones 17:155). In mammals ET is stored in the RBC, nervous system, and liver. In the liver of rodents, mM levels of accumulated ET inhibit lipid peroxidation without affecting measured drug metabolizing enzyme activities or glutathione levels and without observed toxic effects (e.g. Kawano et al. 1983 Chem. Pharm. Bull. 31:1676, 1682). We have found that the imidazole sulfhydryl, ET, differs from the traditional cysteinyl sulfhydryl in that ET is much less readily autooxidizable nor does ET react with N-methyl-N'-nitro-N-nitrosoguanidine. ET at physiological levels strongly blocks the mutagenic action on Salmonella strain TA1950 (hisG46 uvrB) of direct-acting electrophilic mutagens such as some of the multiple products derived from nitrosation of the ubiquitous polyamine, spermidine. Roughly half of the mutagenic activity of nitrosated spermidine is eliminated at 2 mM ET. Antimutagenic activity is proportional to ET concentration over the range tested, namely from 0.1 to 8 mM ET, reaching 90% interception of mutagenic activity at 8 mM. We consider ET to constitute an important natural strategy, possibly applicable to man following ingestion, for blockage of macromolecular attack by a variety of otherwise genotoxic electrophiles. (Supported in part by grant ES03217).

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EVALUATION OF THE MUTAGENICITY OF CRYSTAL VIOLET AND ITS METABOLITES IN SALMONELLA TYPHIMURIUM AND EXCHERICHIA COLI. B.S. Hass, R.H. Heflich and J.J. McDonald, National Center for Toxicological Research, Jefferson, AR 72079.

The human population may encounter crystal violet (CV, hexamethylpararosaniline) in a wide variety of ways since the dye is used in inks and in treating neonates and human blood for the prevention of microbial infection. Several metabolites of CV have been found in the tissues and excrement of rats, mice, and chickens, including pentamethylpararosaniline (PMPRA), N,N,N',N'-tetramethylpararosaniline (d-TMPRA), and N,N,N',-N''-tetramethylpararosaniline (t-TMPRA). A reduced, noncolored form of CV (leuco-CV) and of PMPRA (leuco-PMPRA) also have been isolated. The mutagenicity of the above compounds was determined in S. typhimurium strains TA97, TA98, and TA100. The colored compounds were confirmed as toxic, but not mutagenic, over the concentration range of 1 to 50 µg/plate in TA97 and TA98 in the absence and presence of rat liver homogenate (S9). Strain TA100 also showed no mutagenicity, but the toxicity of the compounds was eliminated in the presence of S9. Leuco-CV and leuco-PMPRA each induced over twice the background number of TA98 (54 revertants/plate) at 50 µg/plate in the presence of S9. In continuous (chemostat) culture, E. coli strain WP2s (trp,uvrA) produced a positive response using phage T5 resistance at 5 µM concentrations of CV (38 mutants/10^8/generation), PMPRA (31), d-TMPRA (89) and t-TMPRA (135). Background was 2 mutants/10^8/generation. Chromatography of spent chemostat cultures using CV and PMPRA showed the presence of the leuco derivatives of CV and PMPRA, respectively. Thus, the leuco derivatives of CV and its metabolites may be the active mutagenic components of these dyes.

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INCREASED ADENOVIRUS(SA7) TRANSFORMATION IN A LINE OF CLONED RAT EMBRYO (CREF) CELLS PRETREATED WITH CARCINOGENS. G. Hatch, L. Rogers*, J. Bilotta*, and T. Anderson*, In Vitro Toxicology, Northrop Services, Inc., Environmental Sciences, Research Triangle Park, NC 27709.

Pretreatment of target cells in culture with chemical or physical agents enhances the production of transformed viral foci following subsequent DNA virus treatment. We have used this approach to examine the ability of a wide variety of mutagenic and carcinogenic chemicals to quantitatively enhance Simian adenovirus SA7 transformation utilizing both primary hamster and continuous lines of rat embryo cells. The objective of this study was to identify a cloned cell line of embryo origin that was sensitive to chemical enhancement of SA7 adenovirus transformation. Such a cloned cell system, if suitable, would offer potential advantages for assay standardization, analysis of molecular mechanisms of action and comparison with other in vitro transformation end points. We report here that a cell line (CREF) at early passage isolated from a single cell clone of the F2408 Fisher rat embryo fibroblast line(Fisher et al PNAS:3527; 1982) is sensitive to SA7 transformation. Virus transformation frequencies($1-10 \times 10^{-}$) are equal to or greater than those ob-